

DTIC FILE COPY

# Comparison of Quantitative Microbiology and Histopathology in Divided Burn-Wound Biopsy Specimens

DTIC  
SELECTED

JUL 24 1987

Albert T. McManus, PhD; Seung H. Kim, MD; William F. McManus, MD; Arthur D. Mason, Jr, MD; Basil A. Pruitt, Jr, MD

• We examined the association between quantitative microbiologic results and histopathologic findings in divided biopsy specimens from 200 burned patients. Microbiologic counts were determined as  $\log_{10}$  colony-forming units per gram of disrupted tissue. Histopathologic results were scored on a scale of 1 to 6, values of 4 or greater indicating microbial invasion of viable tissue. Agreement of 96.1% was found between negative cultures, arbitrarily identified as those with fewer than 5 logs/g, and histologic absence of invasive infection. In sharp contrast, however, histologic invasion occurred in only 36% of specimens with positive cultures. Though low tissue counts are essentially synonymous with negative histologic findings, quantitative microbiology is not a diagnostic substitute for histologic examination, since high tissue counts quite commonly do not indicate invasion. The principal value of quantitative burn-wound biopsies is the demonstration of predominant burn-wound flora.

(Arch Surg 1987;122:74-78)

The burn wound is the most obvious potential source of sepsis for the seriously burned patient. Despite improvements in antibiotics and the development of topical antimicrobial burn treatments, burn wounds commonly become colonized with potentially invasive organisms derived from both endogenous and exogenous sources. Management of burned patients after resuscitation and during the time when wounds are open is, in large part, guided by observations of the condition of the wound. Evaluation of the level of control of microbial growth is a critical factor in instituting further steps to prevent or treat burn-wound sepsis. Several quantitative culture techniques have been proposed as means of estimating the microbial density in burn wounds.<sup>1-6</sup> Densities of  $10^4$ ,  $10^5$ , and  $10^6$  per gram of tissue have all been proposed as diagnostic of burn-wound infection. We examined the correlation between quantitative cultures and diagnostic histologic findings<sup>7,8</sup> in paired samples from burn-wound biopsy specimens from 200 patients.

Accepted for publication June 24, 1986.  
From the US Army Institute of Surgical Research, Fort Sam Houston, Tex.

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

Read before the Sixth Annual Meeting of the Surgical Infection Society, Chicago, April 21, 1986.

Reprint requests to Library, US Army Institute of Surgical Research, Fort Sam Houston, TX 78234-6200.

## PATIENTS AND METHODS

Biopsy specimens for analysis were selected for each patient on the basis of their being the first postadmission diagnostic biopsy specimen submitted for quantitative culture and histologic examination. The demographic characteristics of the 200 patients sampled are presented in Table 1. The mortality of 58.5% documents the severity of injuries in this group. The distribution of postburn times at which biopsies were performed is presented in Fig 1. The average time of biopsy was 13 days after injury, and the median was nine days. Biopsy specimens were divided by the surgeon; one half was sent to the histology laboratory and the other to the microbiology laboratory. Histologic examination was performed on sections prepared by a rapid technique,<sup>9</sup> and results were reported within four hours as infection or no infection on the basis of presence or absence of organisms in viable tissue subjacent to the burn wound.<sup>7</sup> The stage of infection or colonization was scored on the following scale: 1, superficial colonization; 2, penetration of nonviable tissue; 3, growth at viable-nonviable interface; 4, microinvasion; 5, florid or generalized invasion; and 6, microvascular invasion. Grades 1 through 3 represent progressive depths of colonization of nonviable tissue, while grades 4 through 6 represent increasing depth of invasive infection.

Cultures were performed after disruption of the specimens with Ten-Broeck glass homogenizers using tryptic soy broth as diluent. The disrupted specimen was serially diluted, and organisms were counted by pour-plate culture using tryptic soy agar. Colonies were counted after 24 and 48 hours of aerobic incubation at 35°C. The disrupted sample was also plated for fungus isolated by inoculating Sabouraud's agar and incubating for as long as five weeks at room temperature.

Pearson's correlation coefficient and Spearman's rank correlation coefficient were calculated from the paired numeric histology grades and the  $\log_{10}$  colony counts of quantitative cultures.

## RESULTS

Invasive wound infection was identified in 39 of the 200 biopsy specimens. The distribution of negative and positive biopsy specimens as a function of the results of quantitative

Table 1.—Characteristics of 200 Patients Undergoing Biopsy

Variable	Value
Mean age, y (range)	37 (1-88)
Mean total burn area, % (range)	54.1 (6.8-96.5)
Mean total 3rd-degree burn, % (range)	29.3 (0-86)
Mean postburn day of biopsy (range)	13 (1-88)
Sex, M/F	168/32
Mortality, No. (%)	117 (58.5)

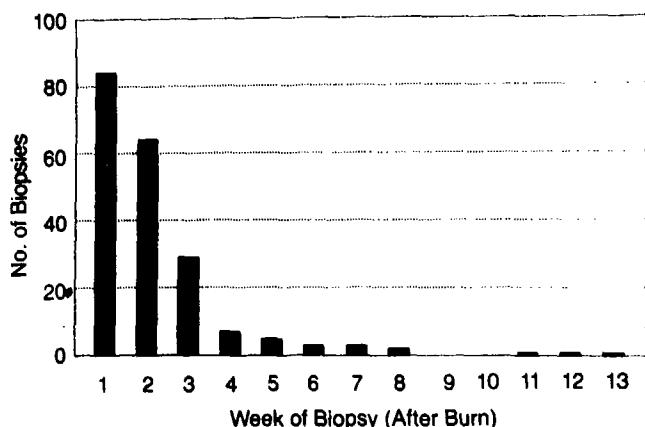


Fig 1.—Frequency distribution of examined burn-wound biopsy specimens by time after injury.

culture is presented in Table 2. Analysis of these data showed a significant correlation ( $r=.59$ ,  $P<.05$ ) between histology grade and log of culture growth. Figure 2 displays these data as the percentage of positive and negative histologic findings at each log count. As can be seen, the greatest correspondence between histology grade and microbial growth was that between negative histologic findings and low counts. At least half of all biopsy specimens were histologically negative for infection at all growth levels less than  $10^9$  organisms per gram.

In an attempt to analyze the data further, we used a frequently quoted yet arbitrary level of  $10^5$  organisms per gram to assign the biopsy specimens to culture-positive and culture-negative sets. These two populations were then examined for the frequency of positive and negative histologic findings. Again, the most comprehensive statement that can be made is that negative histologic findings agreed with growth of less than 5 log units 96.1% of the time (Table 3). In less than 40% of cases was there a histologic diagnosis of infection when the culture density was  $10^5$  organisms per gram or higher. Changing the density of organisms defining infection to either  $10^4$  or  $10^6$  per gram of tissue did not influence the disparity.

The histologic findings of organisms seen causing the 39 wound infections were next compared with the types of organism cultured. There were 29 cases of invasive infection due to gram-negative bacilli, two cases of invasive infection due to gram-positive cocci, and eight cases of invasive fungal infection. Culture results agreed with all histologically diagnosed bacterial infections and five of eight histologically diagnosed fungal infections (Table 4), for an overall accuracy of 92%.

#### COMMENT

The purpose of this study was to determine the extent to which quantitative cultures of burn-wound biopsy specimens are consistent with histologic diagnoses of invasive wound infection. As logic predicts, low levels of microbial growth in tissue were associated with negative histologic findings. Only four instances of invasive wound infection

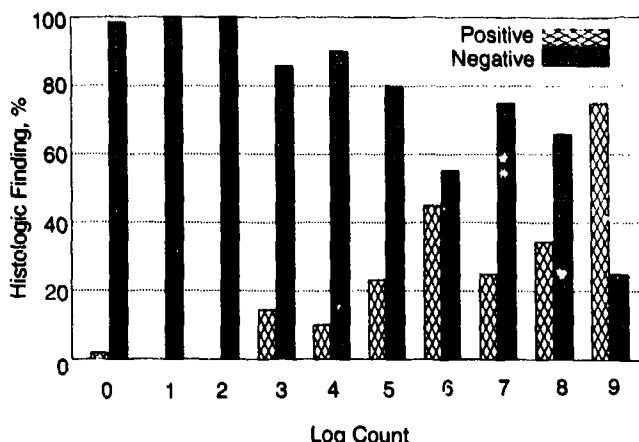


Fig 2.—Frequency of negative and positive histologic findings in burn-wound biopsy specimens by quantitative culture results (log<sub>10</sub> organisms per gram).

Table 2.—Quantitative Biopsy Results vs Histopathologic Findings

Log Count*	Histopathologic Finding		
	Negative	Positive	Total
<1	61	1	62
1	2	0	2
2	11	0	11
3	6	1	7
4	18	2	20
5	10	3	13
6	16	13	29
7	15	5	20
8	21	11	32
9	1	3	4
Total	161	39	200

\*Log<sub>10</sub> organisms per gram.

Table 3.—Positive Culture\* vs Positive Histopathologic Findings

Culture	Histopathologic Finding, %		
	Negative	Positive	Total
Negative	96.1	3.9	100.0
Positive	64.3	35.7	100.0
Total	80.5	19.5	100.0

\*Culture positivity based on an arbitrary criterion of  $10^5$  organisms per gram.

occurred in 102 specimens with microbial densities of less than  $10^5$  organisms per gram; two of these infections were fungal. The antinomy is that high levels of microbial growth were more often associated with negative histologic findings indicative of bacterial growth in nonviable tissue than with invasive wound infection. Thus, it appears that histologic and microbiologic diagnostic techniques yield equivalent information in clean wounds, but that there is no microbial density that, taken alone, permits the diagnosis of

Table 4.—Histopathologic Findings and Organisms Cultured

Histopathologic Finding	Organism Cultured	No. of Cases
Gram-negative rods	<i>Pseudomonas aeruginosa</i>	22
	<i>Klebsiella pneumoniae</i>	4
	<i>Pseudomonas fluorescens</i>	1
	<i>Providencia stuartii</i>	1
	<i>Serratia marcescens</i>	1
	Total	29
Gram-positive cocci	<i>Staphylococcus aureus</i>	2
Filamentous fungi	<i>Aspergillus</i> species	2
	<i>Fusarium</i> species	2
	<i>Rhizopus</i> species	1
	None*	3
	Total	8

\*Not diagnosed by culture.

invasive wound infection. At most, a high density indicates the need for histologic examination. Clearly, these findings indicate that diagnoses of burn-wound infection and therapeutic interventions based on quantitative microbiologic techniques suffer a lack of specificity.

The good agreement between the histologic and microbiologic identifications of infecting organisms shows that cultures should be viewed as an adjunct to histologic examination of a burn-wound biopsy specimen. In the usual clinical situation, therapy for invasive infection is instituted before the results are available. Despite the 24 to 48 hours required to obtain the information, such cultures do provide useful species identifications and information concerning antibiotic and chemotherapeutic sensitivity of the invading organisms. Such qualitative information, however, may not necessarily require quantitative culture technique.

#### References

1. Brentano L, Gravens KL: A method for quantitation of bacteria in burn wounds. *Appl Microbiol* 1967;15:670-671.
2. Georgiade NG, Lucas MC, Osterhout S: A comparison of methods for the quantitation of bacteria in burn wounds: II. Clinical evaluation. *Am J Clin Pathol* 1970;53:40-42.
3. Loeb EC, Marvin JA, Heck EL, et al: The method of quantitative burn-wound biopsy cultures and its routine use in the care of the burned patient. *Am J Clin Pathol* 1974;61:21-24.
4. Levine NS, Lindberg RB, Mason AD Jr, et al: The quantitative swab culture and smear: A quick, simple method for determining the number of viable aerobic bacteria on open wounds. *J Trauma* 1976;16:89-94.
5. Woolfrey BF, Fox JMK, Quall BS: An evaluation of burn wound microbiology: The quantitative Gram stain. *J Burn Care Rehabilitation* 1982;3:171-175.
6. Boyle J, Roberts FJ, Snelling CFT, et al: Rapid, simple, and inexpensive method to rule out bacterial invasion of fat subjacent to full-thickness burn eschar. *J Burn Care Rehabilitation* 1985;6:402-407.
7. Pruitt BA Jr, Foley DF: The use of biopsies in burn patient care. *Surgery* 1973;73:887-897.
8. Pruitt BA Jr: Biopsy diagnosis of surgical infections. *N Engl J Med* 1984;310:1737-1738.
9. Kim SH, Hubbard GB, Worley BL, et al: A rapid section technique for burn wound biopsy. *J Burn Care Rehabilitation* 1985;6:433-435.

#### Discussion

CHARLES E. HARTFORD, MD, Chester, Pa: It has been my long-standing contention that the results of burn-wound biopsy quantitative culture bear no relationship to the clinical condition of the patient and that the results of the quantitative count are of no value in managing a patient. Therefore, I wish to thank the authors for bringing to our attention additional information that drives yet another nail into the coffin of this test.

On the other hand, I concur that the histologic examination of subeschar tissue to determine the presence or absence of invasive infection is of value. This is a reaffirmation of a recommendation made by Pruitt and Foley<sup>7</sup> in 1973. The material in this report is well presented by Dr McManus and his cohorts, not overinterpreted, and the conclusions are appropriate; so I will provide the overinterpretation.

From a historical perspective, one might theoretically consider that microbial counts or microbial densities would have value in predicting burn-wound sepsis. However, the information that substantiates this has not been forthcoming.

It is critically important to correlate bacterial counts with the clinical condition of the patient. However, although not specifically stated in the report, I assume that the reason for performing the biopsy was because the patient was clinically ill from sepsis. If that is in fact the case, and one uses  $10^4$  or 100 000 organisms per gram of tissue as the critical point, then one half of the bacterial counts were positive and one half were negative, and one cannot draw conclusions from this kind of a result.

We looked at this issue as a function of postburn time. We studied a group of patients at high risk for burn-wound infection and systematically did quantitative counts. This display shows that by the end of the first postburn week 80% of the patients had the clinical manifestations of sepsis, but only 15% of them had positive wound biopsy quantitative counts. This relationship has a high degree of statistical significance, with a *P* value of  $5 \times 10^{-5}$ , indicating that the presence of clinical sepsis and a positive biopsy specimen during the first week are not related. The same relationship holds during the second week, but our data suggest that during the third or fourth week there may be a relationship between the clinical condition of the patient and a positive biopsy specimen. Those who advocate the use of quantitative counts maintain that the usefulness of this test is diminished by this time.

There are causes for sepsis in the burned patient other than the wound, and we looked at those patients with inhalation injury. If one separates out those patients with inhalation injury, one would conclude that the major source of sepsis would be the wound. We did quantitative counts in those patients and could also find no difference between those who were septic and those who were not.

I would like to ask Dr McManus to comment on the indications for biopsy, how the sites were chosen, and to describe the patient's clinical condition in reference to the results of not only the quantitative counts but also the histologic results.

DAVID AHRENHOLZ, MD, St Paul: I would like to echo Dr Hartford's questions regarding indications for wound biopsy and ask what the correlation was between clinical sepsis, as evidenced by changes in mental status, hypotension, oliguria, decreasing platelet count, hypoglycemia, and so forth, and results of the burn-wound biopsy. Also, did you begin treatment with antibiotics in patients with positive biopsy specimens who were clinically stable?

DR McMANUS: As I said, these were 200 patients and 200 biopsies, one biopsy performed in each patient. There was no prospective analysis of any patient by use of serial biopsies. I showed you that the mortality for this group was 58%, which indicates that these were serious burns. All of the biopsy specimens presented were for diagnosis. Patients were septic or considered to be questionably septic at the time of biopsy.

The purpose of this was to evaluate these two laboratory tests. We did look at mortality as a function of positive histologic or culture findings as a bottom line. The patients who had positive pathologic findings had a significant increase in mortality compared with patients who did not have positive findings. However, in patients who had a positive culture based on  $10^4$  or  $10^5$  or  $10^6$  organisms, there was no correlation with survival.



A-121